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INVITED REVIEW

Spermine metabolism and radiation-derived reactive oxygen species for future therapeutic implications in cancer: an additive or adaptive response

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Abstract Destruction of cells by irradiation-induced radical formation is one of the most frequent interventions in cancer therapy. An alternative to irradiation-induced radical formation is in principle drug-induced formation of radicals, and the formation of toxic metabolites by enzyme catalyzed reactions. Thus, combination therapy targeting polyamine metabolism could represent a promising strategy to fight hyper-proliferative disease. The aim of this work is to discuss and evaluate whether the presence of a DNA damage provoked by enzymatic ROS overproduction may act as an additive or adaptive response upon radiation and combination of hyperthermia with lysosomotropic compounds may improve the cytocidal effect of polyamines oxidation metabolites. Low level of X-irradiations delivers challenging dose of damage and an additive or adaptive response with the chronic damage induced by spermine oxidase overexpression depending on the deficiency of the DNA repair mechanisms. Since reactive oxygen species lead to membrane destabilization and cell death, we discuss the effects of BSAO and spermine association in multidrug resistant cells that resulted more sensitive to spermine metabolites than their wild-type counterparts, due to an increased mitochondrial activity. Since mammal spermine oxidase is differentially activated in a tissue specific manner, and cancer cells can differ in term of DNA repair capability, it could be of interest to open a scientific debate to use combinatory treatments to alter spermine metabolism and deliver differential response.

Keywords Spermine · BSAO · Radiation · DNA damage · Lysosomotropic compound · ROS

Abbreviations

ADR	Adriamicyne resistant cells
AO	Amine oxidase
APAO	Acetyl-polyamine oxidase
BER	Base-excision-repair
BSAO	Bovine serum amine oxidase
DSB	Double strand break
DX	Doxorubicin resistant
FAD	Flavin-adenin-dinucleotide
H_2O_2	Hydrogen peroxide
IU	International units
LNT	Linear no-threshold
METC	Mitochondrial electron transport chain
MDL 72527	N1,N4-bis(2,3-butadienyl)-1,4-butanediaminedihydrochloride
MDR	Multidrug resistant
NER	Nucleotide-excision-repair
PA	Polyamine
P-gp	P-glycoprotein
ROS	Reactive oxygen species
SMOX	Spermine oxidase

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SPD	Spermidine
SPM	Spermine
SSB	Single strand break
TC-NER	Transcription coupled-NER
TEM	Transmission electron microscopy
JC-1	5,5,6,6-Tetrachloro-1,1,3,3-tetraethylbenzimidazolcarbocyanine iodide
WT	Wild-type

Introduction

A large amount of human cancer have been directly related to degenerative inflammation (Mueller and Fusenig 2004), since first evidences by Rudolf Virchow (Balkwill and Mantovani 2001). Inflammatory bowel disorders, namely ulcerative colitis and Crohn's disease, lead to colorectal cancer (Seril et al. 2003), and the administration of anti-inflammatory drugs reduces tumorigenesis (Eaden et al. 2000). During inflammation and in most chronic diseases the reactive oxygen species (ROS) produced by cellular metabolism are not sufficiently detoxified. Indeed, increased ROS level downstream the inflammatory stimuli are considered chemical effectors in inflammation-driven carcinogenesis (Kundu and Surh 2008). Damaged DNA by oxidative species causes genomic instability and replication errors, both prodromal markers of tumor transformation (Cooke et al. 2003). The biochemical marker of oxidative DNA damage, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) has been found augmented in *Helicobacter pylori*-induced gastric tumor (Xu et al. 2004) and TNF-α-induced pulmonary carcinogenesis (Babbar and Casero 2006). Owing to their chemical reactivity, radicals have cytotoxic properties and have been extensively described as mutagenic, and to cause cell death and apoptosis. Controversially, balanced ROS level may provoke a positives outcome of *hormesis* (Goldman 1996) as demonstrated in human hyper-baric oxygen therapy (Dennog et al. 1996) and in the oxidative damage after γ-irradiation in rats (Zastawny et al. 1997). Thus, both exogenous and endogenous exposures to ROS may in turn play a fundamental pivotal role in cell behavior (Schumacker 2006). Recently, exploiting increased ROS levels and altered redox status in cancer cells has become a new therapeutic strategy to improve cancer selectivity over normal cells (Trachootham et al. 2009). Moderate increases in ROS may confer cancer cells advantages, such as faster genetic mutations, higher cell proliferation and elevated differentiation rates. Numerous studies have demonstrated that in the organisms hydrogen peroxide (H_2O_2), as other ROS, are able to affect cell cycle progression, inducing inhibition of cell proliferation and a block in G1, S or G2 phases of the cell cycle (Boonstra and Post 2004). In eukaryotic cells, the signaling pathways modulated by ROS that control the cell cycle progression are complex. The G0 to G1 transition that leads cells from quiescence to division is the only cell cycle transition that is independent of cyclin-dependent kinases (CDK)–cyclin complexes. H_2O_2 can both halt and promote the cell cycle, while O_2^- has been shown to provoke or prevent apoptosis, depending on the cell type. ROS can thus regulate contradictory mechanisms in a dose-dependent manner. High ROS levels mean high risk of DNA and cellular damage, dealing to suppression of DNA replication, arresting cell cycle and after prolonged arrest, triggering apoptosis (Santiago Diaz-Moralli et al. 2013). The growth arrest can be transient or permanent and, in the latter case, the process may end in cell death by apoptosis or necrosis, depending on the entity of the oxidative stress, time of treatment and cell type. Since the most frequent interventions in cancer therapy are the destruction of cells by radical formation induced both by irradiation, and by metabolic drugs via enzyme catalyzed reactions, in the present mini-review, we discuss the future, hypothetical positive (additive) or negative (adaptive) therapeutic implications for cancer and hyper-proliferative diseases coupling ROS unbalanced effects provoked by radiation exposure and alteration of polyamine (PA) metabolism, mainly due to spermine (SPM) catabolism.

Radiation-induced DNA damage and therapy

Cellular DNA is damaged by ionizing radiation being affected with over 20 types of base damage, single-strand breaks (SSB), double-strand breaks (DSB) and DNA–DNA and DNA–protein cross-links (Eccles et al. 2009). Ionizing radiation is defined by the adsorbed and biological equivalent doses. The adsorbed dose is the amount of energy deposited per unit mass. According to the standard international (SI), the deposition of 1 J of energy per 1 kg of matter equals to 1 gray (Gy). The adsorbed dose does not depend on the nature of the traversed matter. The equivalent biological adsorbed radiation dose is an averaged measure of the incident radiation targeting a fixed mass of biological tissue. It takes into account the different biological damage potential of different types of ionizing radiation. According to the SI system, equivalent dose is defined as sievert (Sv) and equals to the adsorbed dose (Gy) multiplied by a radiation weighting factor proper to the type and energy of radiation. Based on the recommendations of the International Commission on Radiological Protection (Recommendations of the International Commission on Radiological Protection 2012), low linear

energy transfer (LET) ionizing radiation (electromagnetic waves such as gamma particles of X-rays) has a weighting factor = 1 (1 Gy = 1 Sv), meanwhile high-LET radiation (such as protons, neutrons, alpha-particles) has a weighting factor expanding from 1 to 20, depending on the energy. For the sake of clarity and to give a first sight of the framework of social radiation incidence, for those who are not familiar with radiation biology, a background dose of natural radiation exposure is around 3 mSv/year, and the radiation exposure dose-limit is 20 mSv/year. Epidemiological data indicate that 10–50 mSv for an acute exposure and 50–100 mSv for a chronic exposure are increasing the probability to have degenerative disease as cancer (Brenner et al. 2003). Cellular DNA is effectively damaged by ionizing radiation, with about 30–40 DSBs provoked by the exposure to 1 Gy (Ward 1998; Löbrich et al. 2005). Indeed, it was postulated that the initial damage to the DNA within a cell nucleus is fundamental to the cell death (Prise et al. 2005).

Radiotherapy is based on this idea; the exposure to an adequate dose of ionizing radiation kills malignant cells. Since the first experience to treat cancer with radiation, at the end of the nineteen century, there was an increasing interest in understanding the mechanistic pathways mandatory to define the energy deposition in a tumor cell and probability of cell survival (Hall 2000). This effort produced abundant information on DNA damage-mediated mechanisms of cell killing by ionizing radiation, in such an amount to justify the DNA-centric approach, also termed as "targeted effect" (Maxwell et al. 2008). During the past 15 years, a new framework has been constructed to consider the mechanisms that involve complex cross-talk signaling pathways in cells and between cells within tissues (Maxwell et al. 2008; Fratini et al. 2011). These several recently accepted experimental evidences in which biological effects are not directly related to the amount of energy deposited in the DNA of the cells traversed by the radiation have been classified as so-called "non-targeted effects" (Prise et al. 2005; Maxwell et al. 2008). These effects include signaling pathways that are not only nuclei, but also cytoplasmic and membranes located (Prise et al. 2005), and strongly depend upon diffusible factors, such as ROS (de Toledo et al. 2006). Among the "not-targeted effects", adaptive responses (Wolff 1998), low-dose hypersensitivity (Joiner et al. 2001) and the genomic instability (Morgan 2003), represent a valid cross-links between radiation effects and SPM metabolism (Cervelli et al. 2012), and we try to address them into a clinical context (see Fig. 1). The adaptive response can be defined as a mitigation in sensitivity to radiation damage induced by a low dose (priming dose), which is delivered prior to a larger, supposed cellular lethal dose (challenge dose) (Wolff 1998; Sykes et al. 2006). Several evidences reported that ROS may act as a priming dose (Marples and Joiner 1995; Kadhim et al. 2004). From a beneficial point of view, preliminary activation of SPM metabolism could mitigate the deleterious effect of accidental exposure to radiation. Under therapeutic approaches, over exposure of ROS in cancer cells prior to radiotherapy could help tumor cells to survive. Low-dose hypersensitivity has been related to a further resistance to radiation (Joiner et al. 2001). Basically, cell survival curve of irradiated cells is delineated by two distinct tracks. The first track is cell cycle related (Skarsgard et al. 1991, 1994). The second one represents two opposite phenomena: extreme hypersensitivity at very low doses (up to 0.3 Gy) followed by increased radio-resistance at the increasing dose up to about 1 Gy (Lambin et al. 1993; Wouters and Skarsgard 1994). Several arguments propose that increased radio-resistance is an effect of an inducible radioprotective process triggered by a higher level of damage, and therefore cells are hypersensitive to X-ray doses since the delivered damage was insufficient to activate the normally repair-associated mechanism (Day et al. 2006). Altering SPM metabolism and sequentially overactivate ROS production could be of therapeutic advantage in fractionated low-dose radiation protocol, delivering hypersensitivity, and/or used as an alternative to radiation if coupled with chemotherapy. Otherwise, additional damage delivered by both radiation

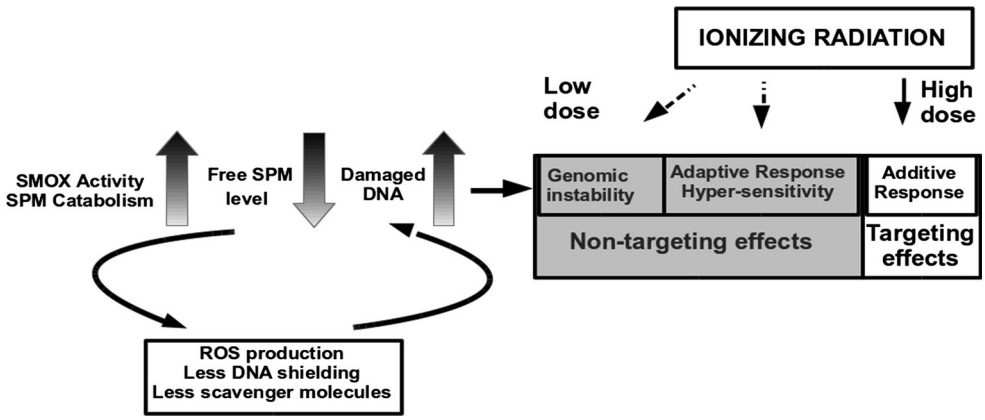

Fig. 1 SPM catabolism and radiation. Schematic representation of relationship between ROS overproduced by SMOX and/or SPM catabolism with ionizing radiation. *Gray shaded* are the non-targeting effects

and SPM metabolism could drive cancer cells into a resistance phenotype. Radiation-induced genomic instability (RIGI) is a non-targeted radiation effect. In progenitor irradiated cells, increased chromosomal instability, apoptosis, and other unfavorable processes affect many generations of progeny (Wright and Coates 2006). RIGI has been strictly related to inflammation, since radiation induces activation of inflammation, which in turn generates ROS and additional DNA damage (Coates et al. 2004). Although cellular normal surveillance for abnormal cells can be activated by low dose of radiation, controversially, radiation may compromise this surveillance itself, thus causing a proliferation and increase of damaged and abnormal cells (Portess et al. 2007). Damaged DNA by oxidative species causes genomic instability and replication errors, both prodromal markers of tumor transformation (Cooke et al. 2003) as well as RIGI. The biochemical marker of oxidative DNA, 8-oxo-dG has been found augmented in *H. pylori*-induced gastric tumor (Xu et al. 2004) and TNF-α-induced pulmonary carcinogenesis (Babbar and Casero 2006). Both of these experimental evidences have been drawn upon SPM catabolism activation and ROS over production.

Spermine oxidase (SMOX) and cancer

Inflammation is the most important nexus recognized between cancer and SPM catabolism. A strong relation links inflammation to several human epithelial cancers via production of H_2O_2 to drive genomic instability. Dealing with SPM catabolism, it has been well defined the negative role played by SMOX (gene deposition reference EC 1,5,3,16, protein deposition references: Q9NWM0 for human and Q99K82 for mouse) and TNF-α activation in human lung epithelial cancer (Babbar and Casero 2006). In prostate cancer, level of SMOX determined by specific antisera reactivity characterizes an early step of tumor progression (Goodwin et al. 2008). The apoptotic activation pathway driven by *H. pylori* provoked mutagenic DNA damage and tumor progression in association with the SMOX activation (Xu et al. 2004). On the contrary, the level of SMOX was inversely correlated to the negative outcome in breast cancer (Cervelli et al. 2010). In breast cancer, SMOX expression and activity were significantly higher in healthy patients, according to the data describing low level of acetyl-polyamine oxidase (APAO) in patients with negative outcome (Wallace et al. 2000). The above described experimental and clinical evidences indicate SMOX as a pivotal player in cancer progression.

SMOX, ROS and radiation in cancer

SPM catabolism is achieved upon the enzymatic activity of the FAD-containing SMOX enzyme. SMOX specifically recognizes SPM as a substrate to produce spermidine (SPD), 3-aminopropanal and H_2O_2 (Vujcic et al. 2002; Cervelli et al. 2003; Polticelli et al. 2012; Cervelli et al. 2013). In several cancer cell lines, the specificity of SMOX to directly oxidate SPM and produce H_2O_2 has been found to be closely related to DNA oxidation and apoptosis (Schiller et al. 2005; Calcabrini et al. 2002; Pledgie et al. 2005) and posed an increased interest in the downstream biological effects provoked by the altered ROS unbalance. As already mentioned, it has been well delineated the capability of ROS to drive cells from proliferation to death (Schumacker 2006), and the metabolic pathways in charge of the PA interconversion and degradation are responsible for the production of the oxidant by-products (Casero and Marton 2007). Since any enzymatic steps of PA metabolism

Table 1 Differential expression of polyamine metabolism genes in hypoxia vs normal conditions (paired *t* test $P < 0.05$)

	GI-LI-N	ACN	SHEP-2	SKNBE(2)c	IMR-32	SKNF-1	LAN-1	SKNSH	GI-ME-N	*P*-test (paired)	Differ. expres.	Fold C. (H/N)	Modul. (1.5)
AMD	1.04	0.79	1.08	0.63	0.60	0.40	1.14	0.85	0.61	0.054	No	0.79	
ODC	0.90	0.74	0.59	0.69	0.81	0.76	0.74	1.01	0.66	0.002	Yes	0.77	
APAO	1.60	1.37	0.72	1.25	0.90	1.48	3.46	1.77	2.11	0.049	Yes	1.63	Up
SSAT	1.01	1.08	1.77	0.97	0.53	0.99	1.09	1.47	1.65	0.090	No	1.17	
SMS	1.19	0.96	0.98	0.98	1.01	1.22	1.33	1.27	1.27	0.078	No	0.82	
SRM	1.16	0.53	0.91	0.73	0.78	0.50	0.81	1.03	0.88	0.026	Yes	1.11	
SMOX	0.60	0.55	0.73	0.28	0.33	0.46	0.84	1.16	0.99	0.012	Yes	0.66	Down

The neuroblastoma hypoxia signature obtained by the discriminatory power of the l1–l2 regularization algorithm and the biological strength of differentially expressed genes were applied (Fardin et al. 2010)

The polyamine gene expression profile was determined on the following genes: *AMD* adenosylmethionine decarboxylase-1, *ODC* ornithine decarboxylase, APAO, *SSAT* spermine-spermidine acetyl transferase, *SMS* spermine synthetase, *SRM* sperimidine synthetase, SMOX

ODC and SMOX were differentially down-regulated

APAO and SRM were differentially up-regulated trespassing the 1.5 threshold of fold change expression

could interfere with ROS-mediated processes, a neuroblastoma hypoxia signature has been determined taking advantage of a previous in vitro experimental model consisting of 11 human neuroblastoma cell lines cultured under normal (20 % O_2) or hypoxic (1 % O_2) conditions for 18 h (Fardin et al. 2010). The hypoxia signature for PA metabolic enzymes was determined in 9 of 11 neuroblastoma cell lines (see Table 1). The only genes statistically significantly trespassing the 1.5 threshold of fold change expression were APAO and SMOX. Interestingly, APAO was up-regulated and SMOX down-regulated, probably due to the hypoxia growth conditions, affecting cell proliferation. However, in the last decade, SMOX activity was found to enhance DNA oxidation in N18TG2 neuroblastoma (NB) cell line, but the consequently induced DNA damage failed to increase cell mortality (Amendola et al. 2013). SMOX overactivity provoked a sublethal chronic DNA damage evidenced by the hyper-phosphorylation of the histone H2AX and subsequent activation of DNA repair, evaluated by the induction of the apurinic/apyrimidinic endonuclease protein-APE1 (Bianchi et al. 2007). Worthy, this sublethal chronic DNA damage did not act as an adaptive response to challenging dose of irradiation, but additive. In fact, delivering 2 and 4 Gy doses of X-irradiation, SMOX-transfected cells were sensitized and more prone to die than mock-transfected cells. Treatments with increasing doses of MDL 72527 [N1,N2-bis(2,3-butadienyl)-1,4-butanediamine], a well-characterized polyamine oxidase inhibitor, abolished such radiosensitive predisposition (Bianchi et al. 2007). However, the level of X-ray delivered has to be considered as high doses, thus belonging to the linear no-threshold (LNT) theory of a linear dose response. Thus, SMOX overactivity should deliver an additive deleterious effect in combination with a high dose of radiation (see Fig. 1). In addition, despite SPM is present in millimolar concentrations in the nucleus, it has been described and characterized with DNA shielding propriety (Rajalakshmi et al. 1980) and as a free radical scavenger (Ha et al. 1998), thus protecting DNA from free radical damage. At the early beginning, Feuerstein et al. (1986) postulated a SPM association with negatively charged molecules such as nucleic acids, resulting in the strongest PA able to stabilize the DNA helical structure. Rajalakshmi et al. (1980) demonstrated that SPM shielded the formation of 7-methylguanine in vitro in rat liver DNA by N-methyl-N-nitrosourea on sites located at, or close to, the binding sites of the ligands, thus affecting the DNA structure and/or its sequences in carcinogenicity driven by DNA interaction. Ha et al. (1988) incubated plasmid circular DNA with ROS generating agents, and discover the inhibition capability of physiologically relevant concentrations of SPM at DSB. Moreover, they demonstrated, by sophisticated analytical and systemic approaches, combining electron paramagnetic resonance, nuclear magnetic resonance, and mass spectroscopy, that SPM is a free radical scavenger. In Fig. 1 these results are summarized and, although it is not fully understood whether the ROS production by oxidative metabolism, or to the depletion of SPM as ROS scavenger and DNA shielding molecule could interfere with cell survival, therapeutic protocols combining enhanced SMO activity with radiotherapy could produce an additive apoptotic effect (Wallace and Fraser 2004; Pledgie et al. 2005).

SMOX, radiation and DNA damage

The radiosensitivities induced by SMOX overactivity in NB cells has been ascribed to an additive response to high doses of X-irradiation in the LNT theory of a linear dose response. At those doses, cells are particularly sensitive to the DSB damages. As few as just a single DBS can trigger the DNA repair processes (Huang et al. 1996). If the DNA repair process fails to fix the damage, just a single DSB can be lethal for the injured cell death (Bennett et al. 1993). Because of this potential lethality, eukaryotic cells have evolved complex and high efficient mechanisms to recognize and repair DSB. There are two main and highly conserved complex repair mechanisms: the homologous recombination (HR) and the non-homologous end-joining (NHEJ) for treating with double-strand breaks and both of them are extensively and better described elsewhere (Jackson 2002). Briefly, these pathways are different and work complementary to each other. Cell takes advantage of undamaged DNA as template to regain genetic information to repair damaged DNA during the HR process. On the contrary, NHEJ does not use any template undamaged DNA but takes advantage of a multifaceted protein repair-associated complex to bring and link together two DSB DNA (Jackson 2002). If the DNA repair process fails to fix the damage, just a single DSB can be lethal for the injured cell (Bennett et al. 1993). In case when the cell is unable to repair completely the DNA damage, misrepaired lesions can lead to DNA mutations (Lobrich et al. 1995). Thus, DSB are crucial, but DNA can suffer for complex injury achieved by group of individual lesions closely localized (0–20-bp) in DNA region. These clusters are not made up of DSB, and can be an aggregation of individual damaged bases, nucleotides and SSB (Goodhead 1994). The mechanisms evolved to recognize and repair such DNA damage are mainly due to the base-excision-repair (BER) (Izumi et al. 2003; Maynard et al. 2009), and nucleotide-excision-repair (NER) (de Laat et al. 1999). BER recognizes and repairs base modifications, abasic sites and DNA SSBs (Bohr 2002). ROS is responsible for many, but not all, of the DNA lesions repaired by BER (Dizdaroglu 2005;

Seeberg et al. 1995). NER is an extremely versatile DNA damage repair pathway activated by the deleterious effects of a large number of DNA noxious agents (Aboussekhra et al. 1995). In this large range, the DNA helix distortion is believed to be a common denominator. Two modes of NER can be distinguished: repair of lesions over the entire genome, referred to as global genome NER (GG–NER), and repair of transcription-blocking lesions present in transcribed DNA strands, referred to as transcription-coupled NER (TC–NER). Making a parallel between DNA damage and the oxidative stress driven by SMOX and/or SPM catabolism, it appears clear that BER should be the principal repair mechanism involved in repairing injury (see Table 2). In fact, previous result demonstrated the APE1 gene involvement in NB cells overexpressing SMOX (Bianchi et al. 2007) and APE1 is a key regulator of the BER process (Izumi et al. 2003). However, oxidative stresses, a non-NER-specific lesion that affect transcription elongation appear to be removed in a TC-NER fashion, linking a blocked polymerase to multiple repair pathways (de Laat et al. 1999). In a recent work, the ectopical overexpression of SMOX coupled with very low-dose X-irradiation (1 and 10 cGy) has been investigated to emphasize the role of the hypothetical priming dose due to ROS overproduced by SPM catabolism and the challenging dose of radiation (Amendola et al. 2013). The challenging doses of X-irradiations were in the low dose range to evidence the influence of SMOX activity, mainly related to alter proliferation and survival, since 10 cGy and less are not delivering any mutation and genomic instability (Maxwell et al. 2008). However, this damage, if not correctly repaired, can provoke genomic instability and increased cancer risk. The chronic sublethal DNA damage driven by SMOX overexpression has been tested in two different DNA repair deficient cellular models, such as EM9 (BER deficient) (Thompson et al. 1982) and UV61(NER deficient) (Thompson et al. 1987) cell lines. According to the DNA repair mechanisms above described, the chronic sublethal DNA damage driven by SMOX overexpression could deliver adaptive or additive response. In NB cell line, SMOX delivers an effect not in agreement with the LNT theory, causing hypersensitivity at lower dose and adaptive response at the higher dose of 10 cGy. In the parental AA8 and BER deficient EM9 cell lines, the priming dose of ROS due to mSMOX, rendered cells less sensitive to DNA damage, thus acting as an adaptive agent. However, since the BER mechanism is the main process to repair DNA damaged by both IR and ROS, at the very low dose of 1 cGy, SMOX was additive to the BER deficiency in EM9 cell line. When the DNA damage is below the threshold to trigger the repair-associated mechanism, cells are hypersensitive to damage, thus explaining these apparently controversial data (Day et al. 2006). When mSMOX was overexpressed in the UV61 cells, the NER deficiently cell line, an earlier adaptive response at 6 h was delivered thus corroborating the hypothesis that SMOX can act as priming, adaptive dose, being NER not primarily involved in those kind of DNA repair (see Fig. 2). All these results are indeed in the very low dose range of exposure and have been detected upon the use of sensitive proliferation and damage assays. When moving to the high dose range, PA metabolism is entirely affected by radiation. Recently, an in vivo experiment demonstrated that SPD is released in serum following 6 Gy of X-irradiation up to 3 days after (Roh et al. 2012). At these range of doses, the whole metabolic effects of radiation should be taken into account. In fact, in the same experiments, the level of SPD paralleled the food consumption and the body weight of experimental animals, and SPD released in the circulating system can be related to the well-known interconversion PA metabolism able to maintain its homeostasis more than related to damaged DNA.

Hyperthermia radiation-derived ROS and lysosomotropic compounds in cancer therapy

Biogenic amines in cell redox balance may behave directly as scavengers against specific types of ROS, or can indirectly cause an increase in ROS production, via H_2O_2 generation, mediated by their oxidative deamination by amine oxidases. These enzymes are important cellular components, since

Table 2 Schematic summary of DNA lesions after ionizing irradiation (IR), ROS and SPM catabolism

Type of damage	Type of lesion	DNA repair system
IR (low dose), alkylating agents	Altered base, abasic site, SSB	BER
ROS, SPM catabolism	Altered base, abasic site, SSB	BER, TC-NER(?)
IR (high dose), alkylating agents	DSB, Intrastrand cross-links	HR, NHEJ
UV light, intercalating drugs	*Intrastrand cross-links, clustered DNA adducts*	*NER*
Replication errors	*Base mismatches, insertions and deletions*	*Mismatch repair*

Italicized are the mechanisms not described in the present mini-review because not relationship between SPM catabolism and DNA damage has been described

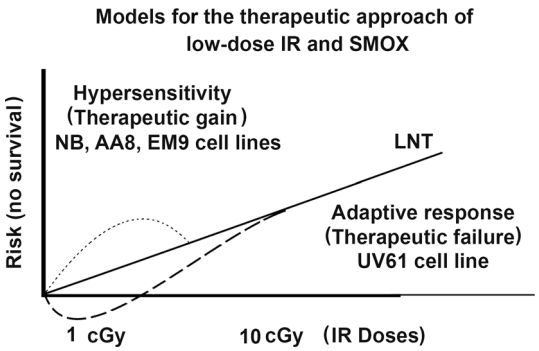

Fig. 2 Models for therapeutical approach of low dose radiation. Models of therapeutic gain or failure targeting hyper-proliferative disease with combinate treatments of ionizing radiation (IR) and activation of SPM catabolism. Symbols and abbreviations: *IR* ionizing radiation, *NB* neuroblastoma AA8, *CHO* parental cell line, *EM9, AA8* defective in BER repair system, *UV61, AA8* defective in TC-NER system, *black line* linear-no-threshold curve of risk, *dotted line* hypersensitivity, *dashed line* adaptive response

they contribute to regulate the levels of polyamines. From a therapeutic point of view, the improvement of the efficacy of in situ formation of cytotoxic PA metabolites is essential. This may be achieved by combinations of the treatment with cytotoxic drugs, or by heat. Hyperthermia is making considerable progress in the cancer clinic (Van Der Zee 2002). In this context, it was observed that hyperthermia potentiates the cytotoxic effects of the oxidation products of SPM and bovine serum albumin (BSAO), H_2O_2 and aldehyde, in several tumor cell lines, in both wild-type (WT) and their multidrug resistant (MDR) counterparts (Calcabrini et al. 2002; Agostinelli et al. 1994, 2006a, b). From this association, it seems that the metabolites of SPM appear to behave as thermosensitizers (Agostinelli et al. 2006a, 2009). It was reported that the combination of hyperthermia with either radiotherapy and/or chemotherapy, led to improved clinical outcome in 18 randomized studies (Van Der Zee 2002). This was demonstrated for melanoma and cancers of the head and neck, breast, brain, rectum and others. Hyperthermia is applied in cancer patients in the clinic by either localized heating of the tumor at temperatures such as 42–43 °C for 1–2 h, or by milder heating at 39.5–41 °C for longer times (6–24 h). Regional hyperthermia has the potential to increase cytotoxic effects of radiation or chemotherapeutic agents within the tumor mass, without increasing normal tissue toxicity. The whole body hyperthermia is often used to treat carcinomas with distant metastases. In this kind of cancer, the cytotoxic effect of aldehydes could be effective. In fact, patients with inoperable carcinomas in terminal stages were treated with aldehyde derivative with satisfactory results (Kochi et al. 1980).

Several findings suggest that the deregulation of polyamine metabolism (putrescine, SPD, SPM and related compounds) may induce apoptosis (Seiler and Raul 2005). Apoptosis is morphologically characterized by rounding up of the cell, retraction of pseudopodia, reduction of cellular volume, condensation of the chromatin, fragmentation of the nucleus, plasma membrane blebbing, but little modification of the cytoplasmic organelles (Kroemer and Martin 2005) and maintenance of an intact plasma membrane. Apoptosis reflects cell responses to physiological and pathological changes of the environmental conditions. In addition to their function in the cleavage of cytoplasmic constituents within the lysosomal lumen, diverse signals are mediated by lysosomal proteinases to caspases, and thus to apoptosis. For instance, radiation-induced cell death involves the formation of ROS that cause lysosomal destabilization and induce caspase activation (Brunk et al. 1997). Since the release of lysosomal cathepsins induces apoptotic and non-apoptotic (necrotic) cell death (Guiccciardi et al. 2004; Stoka et al. 2005), the permeabilities of the lysosomal membrane appear to be a potential target in cancer therapy.

Therefore, for the destabilization of the lysosomal membrane, it seems that two ways of therapeutic potential are available: formation of ROS, for instance by UV or γ-irradiation, or by enzymatic reactions using xanthine oxidase after administration of hypoxanthine (Yoshikawa et al. 1995) or by bBSAO administration in the presence of SPM (Agostinelli et al. 2004).

Currently, we are studying drug combinations with the aim of improving the induction of cell death by toxic PA metabolites. Sensitizing cells by a lysosomotropic compound improved cell damage by H_2O_2 and other SPM metabolites, able to cause apoptotic and non-apoptotic cell death. So, it was demonstrated that the induction of cell death was potentiated by the combined treatments of BSAO/SPM with MDL 72527, a lysosomotropic agent (Dai et al. 1999). Other lysosomotropic compounds that sensitize tumor cells to toxic spermine metabolites, like chloroquine, are being investigated. It seems, therefore, of interest to investigate the chances of lysosomotropic drugs in cancer therapy.

H_2O_2 and enzymatic spermine metabolites induce damage of lysosomal membrane and MDR cells. Sensitization of cancer cells by MDL 72527

Hydrogen peroxide damages lysosomal membranes and initiates apoptosis (Zdolsek et al. 1993). Likewise, H_2O_2 formed in situ, e.g., by enzymatic oxidation of SPM (Agostinelli et al. 2004), is cytotoxic and has antitumor effects in vivo (Averill-Bates et al. 2005). In fact, in vitro studies have demonstrated that the cytotoxic products of BSAO and SPM, H_2O_2 and acrolein can induce either apoptosis or necrosis, depending on both their concentrations and the

cell type (Agostinelli et al. 2004; Teramoto et al. 1999; Lee and Shacter 2000; Kern and Kehrer 2002; Calcabrini et al. 2002). The cytotoxicity was evaluated on both sensitive and resistant human colon adenocarcinoma cells (LoVo WT and LoVo DX, respectively) and melanoma cells (M14 WT and the doxorubicin-resistant line M14 adriamycin resistant (ADR)) by using a clonogenic assay. In the presence of BSAO and SPM, a higher cytotoxicity was observed in LoVo DX than in LoVo WT cells. The percentage cell survival decreased in both cell lines with increasing exposure time. Also M14 ADR cells were more sensitive to the treatment than M14 WT cells. The cytotoxic effect on both cell lines increased as a function of incubation time. It was determined fluorometrically that about 80 % of the formed H_2O_2 crossed the cell membrane, while the aldehyde(s) contributed to cytotoxicity to a lower extent (approx. 20 %) (Calcabrini et al. 2002).

Moreover, it has been demonstrated that pre-treatment of human colon cancer-derived LoVo (Agostinelli et al. 2006c) and M14 human melanoma cells (Agostinelli et al. 2006b) with MDL 72527 sensitizes the cells to subsequent exposure to H_2O_2 and aldehydes generated from BSAO/SPM-induced cell death and potentiates the enzymatic system. The pre-treatment of the cells with MDL 72527 caused the temporary formation of cytoplasmic vacuoles, of lysosomal origin, and also increased the number of lysosomal structures as shown by confocal microscopy studies, when compared with the controls, indicating a contribution of the lysosomotropic properties of MDL 72527 to the sensitization of the tumor cells to H_2O_2 and aldehyde formed during the treatment with BSAO and SPM.

Instead, mitochondrial damage, as observed by transmission electron microscopy (TEM) (Calcabrini et al. 2002; Agostinelli et al. 2006b) seemed to correlate better with the cytotoxic effects induced by the ROS generated during the treatment than with the formation of vacuoles. The mitochondria are the major, though not exclusive source of endogenous ROS. The mitochondrial electron transport chain (METC) activity leads to the formation of ROS such as superoxide radical (O_2^-), H_2O_2 and the hydroxyl radical (HO·) which are usually removed by cells. In essence, the ultrastructural alterations support the view that MDL 72527 acts as a lysosomotropic compound and that the sensitization of M14 melanoma or LoVo colon adenocarcinoma cells to the treatment with BSAO and SPM, as was evident from the decrease of cell survival, is mainly due to the effects induced by pre-treatment with MDL 72527 on the endosomal-lysosomal system, with release of lysosomal enzymes into the cytosol (Agostinelli et al. 2006b, c). The MDR cell lines deriving from LoVo and M14 cells were considerably more sensitive to this treatment than the corresponding WT cell lines. In fact, severe changes of the mitochondrial structure, such as dilatation of the cristae and disruption of membranes, were mainly observed in multidrug resistant cells (Agostinelli et al. 2009). The result represents an important aspect for the application of this strategy, since the occurrence of resistance to cytotoxic agents in cancer cells is one of the most serious obstacles to successful anticancer chemotherapy (Gottesman and Pastan 1993).

Since mitochondria appear to play a pivotal role in determining the differential response between sensitive and drug-resistant cells, a study of the mitochondrial functionality was performed on LoVo cells by flow cytometry. Findings suggested different structural and/or functional properties of the mitochondria present in sensitive and MDR cell lines, that were essential to explain the greater cytotoxic effect caused in LoVo DX cells than in WT ones. Therefore, the cells previously labeled with the probe JC-1, exhibited a hyperpolarization status of the mitochondria of multidrug resistant cells. Since the mitochondrial membrane potential is related to the activity of the mitochondrial electron transport chain (METC), it is supposed that LoVo DX cells can show a higher METC activity than LoVo WT ones. An increased mitochondrial electron transport chain activity was previously observed (Jia et al. 1996) in several multidrug resistant cell lines when compared to their sensitive counterparts. Thus, it was hypothesized that multidrug resistant cells present an increased METC activity because they highly express ATP-dependent P-gp (Jia et al. 1997). The METC activity led to the formation of ROS such as superoxide radical, H_2O_2 and hydroxyl radical (Sohal 1997) which are usually removed by cells (Kowaltowski and Vercesi 1999). An increased basal ROS production in LoVo DX cells was observed, and this could be considered the direct consequence of the higher METC activity in resistant cells when compared to that revealed in LoVo WT cells. The treatments with BSAO/spermine enzymatic system or exogenous H_2O_2 increased the amount of ROS inside the cells. Thus, it could be postulated that multidrug resistant cells resulted more sensitive than LoVo WT cells as they contained a high concentration of ROS which could not be removed by cellular defenses. The accumulation of these molecules induced a higher impairment of the mitochondrial structure and function in LoVo DX than LoVo WT cells. In this experimental model, morphological alterations and the depolarization effect observed in the mitochondria did not represent the typical features of apoptosis (Calcabrini et al. 2002).

Conclusion and perspectives in anticancer therapy

Recently, an increasing interest has been posed on the SMOX and BSAO enzyme activities, since SPM catabolic

degradation has been found closely related to DNA oxidation and apoptosis, mainly via H_2O_2 production (Calcabrini et al. 2002; Arancia et al. 2004). High SMOX activity provokes low level of SPM and the inhibition of the interactions with DNA, thus causing sensitivity to ionizing radiation exposure and cell death. SMOX could deliver a therapeutic gain when forced in NB, parental and cancer cells with impaired BER repair mechanism at low, fractionated dose of IR. Contrarily, in cells with deficiency in NER repair mechanisms, SMOX could play an adaptive role to overwhelm DNA damage by IR and be deleterious for therapy (see Fig. 2). Moreover, it has been demonstrated that cancer cells are selectively killed by hyperthermia alone. Numerous studies evidenced a beneficial effect of hyperthermia when associated with other therapeutic modalities, such as irradiation or chemotherapy, in the treatment of human cancers (Takahashi et al. 1993; Vernon et al. 1996). A considerable enhancement of cytotoxicity at 43 °C was also determined as compared to 37 °C. This led researchers to evaluate the clinical potential of hyperthermia using several temperatures (ranging from 40 to 43 °C) (Hahn 1979). Localized hyperthermia enhances the cytotoxic process of several antitumoral drugs and has considerable potential in cancer therapy (Bates and Mackillop 1990; Dahl 1994). In hyperthermic conditions, greater cytotoxicity was observed in MDR cells than in sensitive cells, in both colon adenocarcinoma and melanoma cell lines. Hyperthermia could act at the initial stage of the treatment, probably by accelerating the kinetics of the membrane molecular interactions and by favoring drug delivery into the tumor mass (Agostinelli et al. 1995, 2006a).

The new approaches show a higher sensitivity to cytotoxic spermine metabolites, H_2O_2 and aldehydes, of MDR human adenocarcinoma and melanoma cells, as compared with their wild type counterparts. ROS formation may become particularly useful in assisting to overcome a major problem of conventional anticancer therapy, namely the development of drug resistance. This finding has been previously attributed to an earlier and higher mitochondrial membrane depolarization, and a higher basal production of ROS (Arancia et al. 2004). In fact, H_2O_2 could directly interact with some iron of Fe/S centers located in the respiratory chain, raising the highly reactive hydroxyl radical (HO·) by means of Fenton reaction, which induces some thiol (SH) groups, proteins and lipids oxidation (Agostinelli et al. 2007). However, cancer cells are also more susceptible to oxidative insults compared to normal cells. Additional ROS stress induced by exogenous spermine metabolite, H_2O_2 and aldehyde, or other agents can overwhelm the relatively low antioxidant capacity and disrupt the redox homeostasis inside cancer cells, which can lead to selective tumor cell toxicity (Gang Huang et al. 2013).

Although still at a beginning, the in situ formation of toxic compounds or radicals by enzyme catalyzed reactions is a promising start. For the slow release of toxic spermine metabolites into the tumour, the use of BSAO conjugated to biocompatible polymers is considered, as previously reported (Averill-Bates et al. 2005; Demers et al. 2001). Treatment of mice bearing subcutaneous melanoma tumors with a low dose of native or immobilized BSAO, induced inhibition of tumor growth by 40 and 70 %, respectively, during a period of 10 days after a single injection of the enzyme into the center of the tumor. The lower *V*max of the immobilized form of BSAO would allow a prolonged, slow release of cxytotoxic products compared to the more rapid generation of higher levels of cytotoxic products with the native enzyme. An important finding is that, under these experimental conditions, the native BSAO generates a burst of H_2O_2 and aldehyde(s) at such concentrations that necrosis is favored, whereas immobilized BSAO caused cell death mainly by apoptosis (Averill-Bates et al. 2005). Although human treatment with BSAO is not yet submitted for being used for future clinical trial in cancer therapy, its use as a model to deaminate PA for generating ROS, demonstrates as these polycations could be administered in clinical applications if produced in presence of amine oxidases isolated from other sources, like plants.

In conclusion, the toxic enzymatic oxidation products generated by BSAO and PA could be useful as a combined treatment with hyperthermia associated with irradiation, or with other drugs, such as lysosomotropic compounds, able to generate ROS. These approaches are of great interest since it might represent a promising strategy to overcome MDR in cancer cells.

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